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NATURE OF FUNCTIONAL RECOVERY FOLLOWING REGENERATION OF THE OCULOMOTOR NERVE IN AMPHIBIANS

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There is a striking difference between the type of motor recovery which occurs in mammals and that which occurs in amphibians following regeneration of divided limb nerves. The abnormal connections formed between central nervous system and musculature by the inevitable misdirection of regenerating motor axons leads in mammals to confused and incoordinate mass contraction of the reinnervated muscles (Sperry, '45c). In amphibians such misregeneration is followed instead by recovery of normal motor coordination (Weiss, '28, '36, '41). Evidently in amphibians the central discharge of the regenerated motor neurons somehow becomes adjusted to suit the particular limb muscles in which the outgrowing axons happen to terminate, but this kind of readaptation does not take place in mammals.

The following experiments were undertaken to find out if the same difference between mammals and amphibians exists with respect to the effects of regeneration of the third cranial or oculomotor nerve. The results of regeneration of this nerve in man have already been amply described (Bender and Alpert, '37, Bielschowsky, '40, Wartenberg, '46) and show that motor readjustment fails to occur, as is characteristic of mammalian nerves generally. There is recovery only of an un-

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differentiated mass contraction of the various muscles supplied by the regenerated oculomotor nerve and this persists permanently without correction. Similar effects have been reported for the chimpanzee and monkey (Bender and Fulton, '38, '39). It only remained for the above purpose therefore to determine whether in amphibians regeneration of the oculomotor nerve would lead to abnormal mass contraction as in man, or whether it would lead to restoration of normal motor coordination as does the regeneration of limb nerves in amphibians.

The problem is significant for a number of reasons. First, the results have important implications regarding the nature of central nervous integration. The recovery of normal coordination despite the mix-up of nerve connections in the limbs has been interpreted to mean that our traditional connectionist theory of central nervous organization is inadequate and must be radically revised (Weiss, '28, '36, '41). It has also been taken alternatively to mean that the normal growth of central synaptic connections is regulated through induction effects from the peripheral end organs (Sperry, '41, '43). In either case the implications are significant and if the latter view is correct, the results assume special interest for their bearing on the ontogenetic development of central reflex associations. The question has relation likewise to problems of reeducation following disarrangement of nerve connections. There is particular interest in the oculomotor nerve in this regard because it is in the ocular motor system that the evidence to date most strongly favors the possibility of immediate spontaneous readaptation of basic neuronal relations (Marina, '15; Leinfelder and Black, '41, '42). The value of advancing the experimental analysis of the vestibulo-ocular system from all possible angles has been pointed out elsewhere (Lorente de Nó, '33; Sperry, '46) and accordingly the data take on added significance by virtue of their application to the motor portion of the vestibulo-ocular reflex arc. Already the analysis of the structure and function of this particular reflex system, contributed in large share by the work of Lorente de Nó, represents the most advanced picture we have of the more refined aspects of central nervous integration.

It was in the hope of gaining further insight regarding the development of appropriate central linkages between the various neuron elements of the vestibulo-ocular system that the following experiments were started. In regeneration of the oculomotor nerve just as in the initial outgrowth in development there arises the problem of adjusting the central and peripheral relations of the individual neurons in a systematic manner so that they become mutually suited to each other and adapted for normal function. The outgrowing axons which terminate in the inferior oblique muscle, for example, must have special functional associations in the nerve centers quite different from those of axons which terminate in the superior rectus muscle or in the medial or inferior rectus muscles, etc. Where such adjustment is attained in amphibians after nerve regeneration, particularly in young larval stages such as are used in the following study, the adjustment presumably involves processes similar to those which operate in normal development.

Previous experiments have shown that the ontogenetic structuring of the vestibulo-ocular reflex mechanisms is accomplished by the growth process itself without aid of learning (Nasiel, '24; Mowrer, '36; Sperry, '46). It has been shown further that in centripetal regeneration of the eighth cranial nerve the different types of sensory fibers establish synaptic relations in the vestibular nuclei in a discriminative manner to suit in each case the particular end organs with which they are connected in the periphery (Sperry, '45b), indicating that the selective linkage of neurons on the sensory side is made possible by differential affinities between the different types of central and peripheral neurons. The present work was begun in search of evidence of the existence of similar specificity and selective affinities on the motor side of this same reflex arc.

PROCEDURE

The plan of the experiments was to sever completely the oculomotor nerve in a rough manner so as to preclude any mechanical guidance of individual fibers back into their original channels. The type of motor recovery which ensued was then to be compared with that known to follow complete interruption of the same nerve in man. The outcome of the initial experiments indicated a necessity for various additional operations and control measures, the nature and purpose of which are explained separately in their contexts.

The compensatory vestibulo-ocular reflexes were used entirely in estimating recovery of function. Amphibians do not make voluntary exploratory eye movements to any appreciable extent nor is the upper lid actively retracted as it is in man. The compensatory eye movements were elicited by tilting the animals in the 3 primary planes of the body. These responses and the method of observing them under a stereoscopic microscope have been described previously (Sperry, '46). It is necessary here, however, to define the terms used below in describing the eye movements in the different body planes. Movement of the eye on its dorsoventral axis in the horizontal plane is referred to as *horizontal movement*. The horizontal rectus muscles, i.e. the medial and lateral rectus muscles, are primarily involved. Movement of the eye around its optic axis is referred to as *wheel movement*. This is effected primarily by the oblique muscles. The eye is said to wheel forward when the dorsal pole of the globe rolls anteriorly and to wheel backward or caudally when the dorsal pole rolls posteriorly. (It should be remembered in visualizing these eye movements that the eyes in these animals are placed laterad.) Movement of the eye around its anteroposterior axis in the transverse vertical plane is called *vertical movement*. Movement in this plane is effected primarily by the vertical rectus muscles, i.e. the superior and inferior rectus muscles. The vertical movements are most pronounced, the horizontal least so.

The extrinsic eye muscles supplied by the oculomotor nerve in amphibians and paralyzed by its severance are: the inferior, superior, and medial rectus muscles and the inferior oblique. The superior oblique muscle was also routinely paralyzed in the main experimental groups by excising a long stretch of the trochlear nerve. This left unparalyzed only the lateral rectus and retractor bulbi muscles and thus virtually abolished all wheel and vertical movements of the eyeball. Any consistent recovery of either vertical or wheel movements in the proper direction as a result of regeneration of the third nerve under these conditions would therefore mean either the presence of selective factors operating in regeneration or else some type of subsequent functional readaptation neither of which appears in man or the chimpanzee.

The animals were frog tadpoles (*Rana clamitans* and *R. grylio*) and salamander larvae (*Amblystoma talpoideum*) gathered in northeast Florida in the spring and early summer. Unless otherwise indicated the tadpoles were in mid- and late larval stages ranging from about 3 to 6.5 cm in length. The amblystoma were also in mid- and late larval stages ranging from 4 to 7 cm in length. The eye movements are decidedly more conspicuous in the tadpoles than in the salamander larvae and somewhat more pronounced in *R. grylio* than in *R. clamitans*.

Anesthetization was carried out in a 2.0% water solution of urethane and the operations were done in a 0.5% water solution of urethane. In the tadpoles a tongue-shaped flap of skin was cut free and folded forward off the cranium. After operation the incision was closed by simply folding this flap of skin back into position. In the urodeles a straight longitudinal incision was made, the edges of which were later approximated as far as possible. The cranial wall over the mid-brain was punctured and broken away in pieces with no attempt to replace it. A fine hook the vertical movement of which was governed by a screw was employed for retraction of the optic lobe. A small suction pipette was used to clear away any blood which escaped into the operating field. Unless other-

wise mentioned the nerves were divided intracranially. They were not cut but were purposely pulled, pinched, and teased apart with fine pointed forceps in a way that left the ends of the nerve stumps frayed, ragged, and well separated. There was no chance under these conditions for the different fiber types to be routed back by mechanical factors alone into their proper pathways in the distal stump. Other technical details having more restricted application are given in their specific contexts.

OBSERVATIONS

Group 1. Oculomotor nerve broken, trochlear nerve widely excised

Almost the entire intracranial length of the trochlear nerve was extirpated. The oculomotor nerve was then divided about halfway between its exit from the brain and its passage through the cranial wall. Twenty-three animals were operated upon, 16 *R. grylio* and 7 *R. clamitans.*

The immediate functional effect of this operation, which paralyzes both oblique muscles plus the superior, inferior, and medial rectus muscles, was to eliminate all vertical and wheel movements of the eye. Only movements in the horizontal plane remained and their excursion was reduced. These horizontal movements were mediated by the lateral rectus muscle the innervation of which remained intact. The unopposed tonic action of this same muscle caused the resting posture of the eye to be deviated temporally.

The first signs of functional recovery occurred about the fifth day after operation when wheel movements began to reappear. These were not normal wheel movements, however, because they were elicited by stimulation which in the normal animal produces only vertical movements nor did they occur in response to stimulation appropriate for wheel movements. Tilting the animals on their longitudinal body axis in either direction from the resting position caused the eye to wheel caudalward on its optic axis. As the head was returned to the level resting position, the eye rolled rostrally again. In the

next few days these wheel movements became stronger and in addition a horizontal component entered the response in that the nasal pole of the eye pulled into the orbit slightly as the eye wheeled caudally.

The above type of reaction, i.e. wheel movement in the caudal direction accompanied by some medial deviation, is just what would be expected from mass contraction of the oculomotor musculature. One would anticipate the wheel component of the reaction to be most conspicuous because the muscle primarily opposing this movement, the superior oblique, had been completely paralyzed by extirpation of the fourth nerve. The medial deviation would be expected to be present but less strong than the wheel component because the action of the muscle responsible, the medial rectus, was opposed by the normal tonus of the intact lateral rectus and also because the normal excursion of movements in the horizontal plane is relatively small. Little or no vertical component was evident in the response. This would be anticipated because the antagonistic actions of the superior and inferior rectus muscles would tend to cancel each other.

Rotation of the animals on their transverse body axis or rotation on their dorsoventral body axis was not so effective in eliciting the mass reaction. This may be attributed to the fact that these stimuli do not normally activate nearly so large a percentage of the oculomotor fibers as does rotation on the longitudinal axis. In some cases a slight caudally directed wheel movement could be elicited by rotation on the transverse axis but this was relatively slight compared to the response to rotation on the longitudinal axis. In the younger animals the abnormal wheel reaction was less marked, perhaps in part because the eye in these cases was already wheeled somewhat caudally in its resting posture.

Between the eighth and fifteenth days a vertical component began to be evident in the response to rotation on the longitudinal axis. The eye deviated downward when its side of the head was tilted upward and vice versa as is normal. These reactions, unlike the above, were selective and adaptive in

nature. The response was not always in the same direction but was directed dorsally when the head was tilted ventrally and ventrally when the head was tilted dorsally from the resting position. The excursion of these properly directed vertical movements, however, was consistently less than normal with considerable variation in different individuals. In some cases the vertical response remained so slight as to be just discernible while in the other cases it became conspicuous and approached about half the normal excursion. There was a tendency for the vertical movements to be recovered a little more completely in the younger larvae and for their recovery to be better in *R. clamitans* than in *R. grylio*.

An inward deviation of the dorsotemporal pole of the eye eventually appeared also in most cases when they were tilted tail up on the transverse body axis. This reaction was found to be present in animals with both oblique muscles denervated and was therefore considered to be further indication of proper action of the rectus muscles.

There was not much additional improvement in the ocular reactions beyond the stage of recovery reached at the end of the third week although half of the animals were kept until metamorphosis. After the third week the eyeball in the majority of cases began to sink into the orbit gradually as if pulled medially by constant contracture of the reinnervated muscles. There was a correlated reduction in the excursion of the ocular reactions. Because the excursion of the eye movements in anurans becomes greatly reduced at metamorphosis no attempt was made to follow the recovery of function beyond this point.

In 4 tadpoles the regenerated third nerve was resected following the recovery of adaptive vertical movements. This again abolished all vertical and wheel movements of the eye, normal as well as abnormal, leaving only horizontal movements mediated by the intact lateral rectus muscle. This ruled out any possibility that the ocular movements attributed to regeneration of the third nerve might have been due instead

to adjustments in the residual function of the lateral rectus and retractor bulbi muscles.

In 5 animals which were prepared for histological examination by the Bodian method it was possible to trace the regenerated third nerve peripherally into the oculomotor muscles. Exact fiber counts were not made but the number of fibers in the regenerated nerve distal to the region of transection was approximately the same as or only slightly less than that in the normal nerve of the opposite side. There was no extreme tangle of fibers in the scar region nor signs of excessive branching. It was difficult, in fact, to determine exactly where the nerve had been divided.

To summarize, the results in this group of animals indicated that the misregenerated fibers, for the most part, had retained their original central reflex timing without adjustment to the new motor terminations. This would account for the abnormal wheel movement with medial deviation and for the gradual retraction of the eye into the orbit, all of which suggest mass, undifferentiated contraction of the oculomotor musculature like that found to follow regeneration of the divided oculomotor nerve in mammals. There was evidence however, in the partial recovery of movements in the vertical plane of an abortive tendency for selective restoration of proper muscle coordination such as is found to follow regeneration of limb nerves in amphibian larvae.

There are several reasons why one might expect an incomplete functional readaptation, if present, to show up better in vertical movements than in either the wheel or the horizontal reactions. In the first place the normal excursion of the vertical movements is greater than that of the wheel and especially that of the horizontal movements. Secondly, there are 2 oculomotor muscles involved instead of 1 with the action of each tending to reinforce that of the other, so that the chances of slight partial recovery becoming manifest in the vertical plane are thus double those in the other planes on this count alone. Finally, the mass action of these 2 muscles would tend to be cancelled out because the 2 are antagonists. This

would permit any minor action in the correct direction superimposed upon the mass contraction to register an observable effect. On the other hand, the mass contraction of the inferior oblique and of the medial rectus muscles was not opposed by any muscle reinnervated by the oculomotor nerve, that of the medial rectus being opposed only by the normal tonus of the lateral rectus, while that of the inferior oblique remained without any opposition whatever.

Group II. Cross union of the trochlear to the oculomotor nerve

To test further the deduction that the misregenerated fibers retain their original timing as indicated by the results in group I, the central end of the divided fourth nerve was crossed to the distal end of the divided third nerve. Under these conditions the oculomotor muscles should become reinnervated by fibers of the superior oblique muscle and should thereafter contract in mass whenever the superior oblique normally responds. One would anticipate the mass reaction to be weak because there are so few fibers in the fourth nerve. Even so it seemed possible that the action of the inferior oblique muscle with no opposition to overcome might register observable wheel movement. Accordingly the operation was carried out in 16 tadpoles, 10 *R. grylio* and 6 *R. clamitans*. The oculomotor nerve was broken where it enters the midbrain and a short length of the stump was removed. The trochlear nerve was broken as far distally as possible and the end pinched against the end of the oculomotor nerve. The ends of the 2 nerves were held thus in contact or close together until they became fixed in place by coagulation of the surrounding media.

The immediate paralytic effects of this operation were similar to those of the preceding series. Between the sixth and eleventh day after operation 11 of the animals began to display wheel movements in the reverse direction from normal. When the tadpoles were rotated head up on their transverse

body axis, the eye rolled backward in its orbit instead of forward as in normal animals. And conversely, as the head was lowered the eye rolled forward again. Some wheel movement also occurred when the animals were rotated on their longitudinal body axis. The eye rolled forward when its side of the head was tilted upward and the eye rolled backward when its side of the head was tilted downward. Slight medial deviation apparently from action of the medial rectus muscle was also noted in most of the cases when the eye rolled backward in response to rotation on the transverse body axis. In the remaining 5 animals the nerve cross was apparently unsuccessful for there was no appearance of reversed movements. In 3 of them weak movements similar to those obtained in group I began to appear after a delay of 9 to 14 days as if the third nerve fibers had succeeded finally in regenerating into their own distal stump.

In 2 of the successful cases the reversed wheel movements persisted with little change. In the others the excursion of the wheel movements diminished gradually after about the tenth day due apparently to conflicting mass contractions resulting from regeneration of the oculomotor nerve which was only delayed rather than completely blocked in these cases. Severance of the regenerated third nerve without damage to the crossed fourth nerve in 2 of them resulted immediately in release again of excellent wheel movements in reverse. Later severance of the crossed fourth nerve in these 2 cases abolished the reversed wheel reaction. The reversal in the timing of the inferior oblique after its reinnervation by fibers from its antagonist in these cases was further evidence that fibers which have regenerated into foreign muscles may retain their original central excitation phase.

The persistence in this group of wheel movements in reverse direction shows that in the amphibians at least the central coordination patterns for eye movement are not immediately readapted to suit extreme peripheral rearrangements as implied by experiments on the transposition of eye muscles in the monkey (Marina, '15; Leinfelder and Black, '41, '42).

Group III. Branches of nerves III, IV, and VI broken separately in the orbit

It is contended by Wartenberg ('46) that the primary cause of mass movements following nerve regeneration is not the misdirection of regenerating fibers as commonly supposed but rather the damaging effect of the peripheral trauma on the central coordinating mechanisms. Division of the individual branches of the oculomotor nerve in the orbit in such a way that the regenerating fibers reach their own distal stumps rather than channels to foreign muscles should and does lead, according to Wartenberg, to mass movements just as does more proximal division with fiber misdirection. The present group of cases was prepared as a test of this point and also as a control for the preceding series to find out if the ocular muscles could function properly after regeneration of the third nerve, provided proper connections were restored. In 12 animals, all *R. grylio*, the roof of the orbit was opened widely and the individual nerve branches to each of the 6 main ocular muscles was divided separately producing complete immobility of the eye.

The results of nerve regeneration in these cases were strikingly different from those obtained in the first series in which there was opportunity for fiber misdirection. Between the fourth and eighth days following operation excellent ocular movements in all 3 planes had been reestablished in 7 of the 12 cases. In the extent of the excursion and in direction and timing the movements approached very closely those of the normal animal. The remaining 5 cases showed good recovery of movement in 1 or 2 planes with only partial recovery in the others, indicating that in these few instances the regenerating axons had not succeeded in reaching their appropriate distal stumps. The rapid restoration of adaptive movements with their full excursion in this group demonstrated that excellent functional recovery occurs when the regenerating nerves terminate in their proper muscles. These results in correlation with those of the preceding series are opposed to the contention of Wartenberg and support the traditional assumption that the mass movements following oculomotor nerve regeneration are attributable to fiber misdirection.

Group IV. Third nerve broken, fourth nerve widely excised in Amblystoma

On the chance that functional readaptation might be more complete in urodeles, the tissues of which tend generally to maintain a greater embryonic lability than those of anurans, the oculomotor nerve was broken in a series of salamander larvae. The operations were performed through a single longitudinal incision; otherwise the surgical technique was much the same as that described for the tadpoles including extirpation of most of the intracranial length of the trochlear nerve.

The type of recovery produced by regeneration of the oculomotor nerve in these urodeles was very similar to that observed in the tadpoles with respect to wheel and vertical movements. Movements in the horizontal plane were not of sufficient magnitude to be tested, even in the normal salamander. The outcome in the urodele differed from that already described for the tadpole in 2 respects: First, the normal excursion of the adaptive vertical movements was more completely restored. The recovery was better in a relative sense only, for the normal excursion of these eye movements in the urodele is much less than in the tadpole. Secondly, wheel movements of almost normal character also were recovered in 7 of the 11 animals. This was apparently due, however, not to the recovery of function of the inferior oblique muscle but instead to regeneration of the trochlear nerve to the superior oblique muscle. Splitting the midbrain roof in the region of the decussation of the fourth nerves abolished the recovered wheel movements in 5 of the animals so treated while leaving reduced wheel movement on the contralateral side where the third nerve had remained intact. It is remarkable that the regenerating fibers of the fourth nerve were able properly to

span so great a distance and arrive correctly at their former termination. As in the tadpoles resection of the regenerated third nerve again abolished the recovered movements in the vertical plane. The tendency toward restoration of proper motor function may have been more pronounced in these animals than in the tadpoles as far as the vertical rectus muscles are concerned. Recovery was not noticeably better, however, in the case of the inferior oblique muscle. The same abnormal wheel component in the response to rotation around the longitudinal body axis appeared and persisted without correction. It was evident that in urodeles as well as in anurans, oculomotor fibers which have misregenerated into a foreign muscle may excite the new muscle without an adaptive change in the central timing of their discharges.

Group V. Regeneration of the oculomotor nerve in very young tadpoles

Since the functional readjustment which follows nerve regeneration in amphibians appears to be essentially developmental in character, the chances of obtaining such functional readaptation should be better, the younger the animals. Accordingly the experiments were repeated on a group of 9 very small tadpoles (all *R. grylio*) about 18 mm in length. The compensatory ocular movements were already present at this stage although their excursion was still relatively small. As in groups I and IV the third nerve was broken and the fourth nerve excised intracranially. A single longitudinal incision was used as in group IV. The fourth nerve was so fine in these very young animals that it was not seen in most cases. It was broken by passing forceps through its pathway between the brain and cranial wall but to what extent the attempt thereafter to extirpate the intracranial length of the nerve was successful remained in doubt.

The outcome in these younger tadpoles differed markedly from the results of the same operation in the older larvae. Complete recovery was eventually attained in all cases. The

period of paralysis and the early stages of recovery during the first 2½ weeks after operation were not much different from the same in the older animals except that the abnormal wheel movements were less pronounced. Unlike the older cases, however, these animals continued beyond the third week to exhibit gradual improvement in the ocular reflex movements. In the course of 7 weeks during which time the animals grew to a length of about 40 mm the reestablishment of the ocular movements in all 3 planes had become complete.

When the cranium was reopened under anesthesia at the end of 8 weeks, the fourth nerve as well as the third was found to have regenerated and both were quite normal in appearance. Resection of the fourth nerve in 2 of the animals decreased the excursion of the wheel movements only partially showing that the reinnervated inferior oblique muscle had recovered its proper function. Resection of the regenerated third nerve later in the same 2 animals produced the same results as after section of the nerve in normal animals, as described under group I. In 2 cases in which denervation of the lateral rectus muscle was satisfactorily achieved there was a survival of weak movement in the horizontal plane. This, plus the excellence of the recovered horizontal movements and the eye posture in general indicated that the medial rectus muscle along with the vertical recti and the inferior oblique had recovered normal function.

Before concluding that the age difference between these animals and those of group I was the primary cause of the difference in results, a control series of 8 older tadpoles was prepared in which the divided fourth nerve as well as the third was left in a position where it could regenerate readily into its own distal stump. Except that adaptive wheel movements were restored beginning about 5 days after operation the results were very similar to those in group I. It was quite clear therefore that regeneration of the fourth nerve could not have been the factor responsible for the excellence of recovery in group V.

The developmental period during which it is possible to sever the oculomotor nerve and still obtain complete motor recovery is evidently a short one. When the experiments were repeated on an additional 9 tadpoles *(R. grylio)* between 24 and 29 mm in length, the type of recovery in even the smallest of these was much like that observed in group I, even though the animals were kept as long as 4 months during which time they doubled in length.

Apparently, in summary, if the tadpoles are young enough when the oculomotor nerve is divided, there is complete restoration of motor coordination. This is consistent with the type of recovery found by Weiss ('36) to follow the regeneration of limb nerves. It must be remembered, however, that in the oculomotor system this type of recovery is confined to very early larval stages whereas in the limbs it is obtained throughout the larval period and even to some extent after metamorphosis. In what measure the underlying mechanism of adjustment in the 2 cases may be similar remains a problem, for in the oculomotor system there is a complicating factor, namely, the addition of a large percentage of new fibers through rapid growth during the recovery interval.

DISCUSSION

With the exception of group V, the foregoing results resemble more the kind of motor recovery which occurs regularly in mammals than that heretofore found in amphibian larvae. Unlike the limb motorneurons of developing amphibians (Weiss, '36, '41) those of the oculomotor system are apparently able to establish and to maintain functional relations with foreign muscles without undergoing adaptive readjustment in their central timing. This was shown by the failure to recover normal coordination and by the persistence of adverse movements indicative of mass contractions in groups I and IV where there was opportunity for extensive misdirection of nerve fibers. It was perhaps most evident from the establishment and persistence of reversed wheel movements in group II following reinnervation of the inferior oblique muscle by nerve fibers of the superior oblique. A definite distinction must therefore be made between the limb and the oculomotor system with regard to the nature of functional recovery following motor nerve regeneration.

That the mass movements in these cases were due actually to the misdirection of regenerating fibers was evident from the contrasting excellence of motor recovery in group III where conditions otherwise were comparable but fiber misdirection was absent. The traditional assumption that the mass movements which commonly follow regeneration of the facial and oculomotor nerves result from fiber misdirection has been challenged recently by Wartenberg ('46). On the basis of clinical observations he has inferred that they must be due instead to a centripetal spread of the effects of the peripheral trauma which in the centers is presumed to cause a selective breakdown of the more refined, and phylogenetically more recent, coordinating mechanisms and consequently a reversion to a more primitive type of coordination. Unfortunate errors and omissions in the argument as well as contradicting evidence such as the above prohibit any serious consideration of this new hypothesis as it stands.

Only a tendency toward the type of motor readaptation characteristic of limb nerve regeneration in amphibians was seen in groups I, II, and IV in the partial restoration of adaptive coordination of the superior and inferior rectus muscles. Although this recovery of properly directed and properly timed vertical movements was only partially complete, its consistent appearance indicated the existence in the recovery process of some factor favoring systematic reestablishment of correct over incorrect movements. On a chance basis alone, the muscles would have had opportunity after regeneration to be excited as much by antagonist nerve fibers as by their own. Some discriminative agency must therefore have been operative to account for the differential dorsal and ventral deviation of the eye in response each to its appropriate stimulation.

There are 3 general ways possible by which, individually or in combination, these differential responses could be accounted

for: (1) In the reestablishment of peripheral connections formation of proper nerve-muscle relations may somehow have been favored over formation of improper connections. (2) With the structural linkages established, the function of the normal nerve-muscle connections may have been favored over that of the abnormal ones. (3) Motor neurons that had made inappropriate terminations may have undergone adaptive readjustment in the timing of their central discharge. The possibility that the learning process may have been involved may be disregarded. Evidence bearing on this point from many sources is overwhelmingly against it. In the present results the lack of variety in the course of recovery in different animals, the persistence of maladaptive wheel movements, and the survival of the movements after decerebration in 5 cases so tested add confirmation to the conclusion that tadpoles are not capable of learning motor reorganization of this sort. Even in man the incoordinate mass contractions persist indefinitely without correction by reeducation.

It has been inferred that (3) above is primarily responsible for readaptation in the limbs of amphibians (Weiss, '36, '41), but there is little in the present evidence to indicate directly what the correct answer may be for the oculomotor system. Nevertheless, in weighing the various possible explanations it is important to note that all of them demand in one way or another the assumption of motor neuron specificity, i.e. the assumption of some constitutional, perhaps biochemical, property by which the motor neurons belonging to the superior and inferior rectus muscles may have been distinguished from each other.

The complete restoration of normal function in group V was more consistent with the results of nerve regeneration found elsewhere in amphibians. Analysis of the results is complicated, however, by the fact that the animals grew rapidly during the recovery period doubling their length and at the same time adding new fibers to the regenerated oculomotor nerve. Nerve regeneration was thus combined in

these cases with initial outgrowth of new motor axons. It is possible that this addition of new fibers was an important factor in the recovery process along with the relatively plastic embryonic condition of the oculomotor system at this early stage of development.

It is necessary in group V as well as in groups I and IV to assume myotypic specificity of the motor neurons in order to account for the orderliness of recovery. Conceivably the original establishment of central-peripheral relations could have been achieved by orderly topographical and temporal relations in the developing embryo. After the nerve trunk had once been broken, however, leaving a gap in the connections unbridged by any discrete pathways in the intervening substrate, no conceivable method remained by which the functional order could have been restored if it be presumed that the nerve fibers were all alike and remained so. In order that the peripheral and central relations of the individual oculomotor neurons, including the new ones as well as those originally severed, could become mutually adjusted in a systematic adaptive manner there must necessarily have been some means by which the nerves for different muscles could have been distinguished from each other.

Exactly how such neuron specification is acquired and how it becomes effective in regulating the reestablishment of functional relations can only be guessed at present. The explanation suggested by Weiss ('36, '41) to account for the orderly recovery of function following regeneration of limb nerves would be applicable to these results. Accordingly it might be supposed that the muscles impose specific biochemical properties on the axons which grow into them and that this produces a selective sensitization of the nerve cells to particular types of central excitatory agents. The Resonance Principle of central nervous function assumes that the motor neurons are bombarded centrally by excitations for all the muscles supplied from the same nucleus but each responds selectively to only a particular one of the various modes of excitation because of its specific biochemical sensitization.

Organized motor function thus becomes possible on this basis despite the disarrangement of nerve connections in the periphery.

There is an alternative possibility for explaining the recovery of function both in the limb and oculomotor systems which assumes that the rearrangement of peripheral connections in regeneration is followed by a compensatory readjustment in the central connections (Sperry, '41, '43). This scheme involves only an essentially minor modification of that proposed by Weiss but the neurological implications are important because it obviates the necessity for any radical revision of the traditional theory of central nervous integration. The specification of neurons by their end organs — evidence for which has been found in the trigeminal (Weiss, '42), optic (Sperry, '43–'45a; Stone, '44) and vestibular nerves (Sperry, '45b) as well as in the limb and oculomotor nerves — is conceived in this hypothesis to be an important factor both in regeneration and in normal embryonic development, in regulating the formation of central synaptic associations. For example, the nerve cells supplying the superior rectus muscle would acquire synaptic linkages different from those supplying the inferior oblique because of special affinities and incompatibilities between the different motor neuron types and the various kinds of central neurons. These affinities and consequently the pattern of synaptic relations in the oculomotor nucleus, presumably are altered when the nerves regenerate into strange muscles provided that the neurons are still in a sufficiently plastic embryonic condition to permit respecification. The new fibers added in the course of growth could become myotypically differentiated either by the muscles into which they are guided or possibly by more proximal or central contacts with neurons already differentiated. At present either of these explanations must be considered tentative in application to the foregoing results until other possibilities incorporating phenomena under (1) and (2) above have been excluded.

The extent of readaptation after nerve regeneration in the amphibian oculomotor system turns out to be intermediate between that observed in the amphibian limb and that found in mammals. This conforms with the theory that the readjustment process is dependent upon an embryonic plasticity and reversibility of neuron specification. The motor ocular nuclei appear much earlier in vertebrate evolution than the limb centers and in ontogeny they show an earlier and more distinct central differentiation. It would therefore be consistent that they should attain more quickly and completely a state of irreversible specificity. Even the limb motor neurons tend eventually to lose the capacity to shift their myotypic specificity in the adult anuran (Weiss, '36). Whereas in mammals the specificity in the motor neurons of the limb as well as of the oculomotor system presumably becomes irreversibly determined very early in development.

There may be a tendency in the course of evolution for the specification of the nerve centers and the adjustment of their connections to become more and more independent of peripheral regulation wherever possible. If so, this would account further for the difference between amphibians and mammals and between the limb and oculomotor centers in respect to recovery following nerve regeneration. Unlike the motor pools for the limb muscles (Romanes, '41), those for the extrinsic ocular muscles undergo discrete self-differentiation before their axons reach the muscles (Neal, '14), and proper peripheral connections can apparently be preassured to the third, fourth, and sixth cranial nuclei by orderly topographical relations and other factors without need for inductive regulation from the periphery. It is only within the field of the third nerve nucleus itself and its system of peripheral terminations that such regulation would appear to be necessary for economy in development. Conceivably the nucleus might differentiate centrally into its constituent motor pools and thereafter the fibers of each pool might seek out their separate and proper part of the primordial muscle mass in the periphery. It would seem more economical, however, for the nucleus to send

its fibers into the muscle mass indiscriminately allowing specificity of the neurons and their central relations to be indueed subsequently through differentiation of the myotome into its separate muscle components.

SUMMARY

1. After intracranial division of the oculomotor nerve in tadpoles and salamanders in mid- and late larval stages, regeneration resulted in persistent mass action of the reinnervated extrinsic ocular muscles indicating that those fibers which had misregenerated into foreign muscles had, in the main, failed to adapt their central reflex relations to suit the new peripheral terminations. Only a tendency toward systematic restoration of adaptive function appeared in the recovery of weak but correctly directed movements of the eye around its anteroposterior axis, dorsally and ventrally each in response to appropriate stimulation.

2. In tadpoles in mid- and late larval stages the branches of the oculomotor nerve were divided separately in the orbit under conditions which allowed the regenerating fibers to restore connections with their original muscles. Under these circumstances regeneration of the oculomotor nerve resulted in an excellent recovery of adaptive motor coordination.

3. In tadpoles of mid- and late larval stages the central end of the divided trochlear nerve was crossed to the peripheral end of the divided oculomotor nerve. The consequent reinnervation of the inferior obique muscle by fibers belonging originally to the snperior oblique muscle resulted in wheel movements of the eye in the reverse direction from normal. This provided further evidence that in amphibian larvae oculomotor nerve fibers can establish functional connections with foreign muscles without undergoing adaptive readjustment in their central reflex relations.

4. After intracranial division of the oculomotor nerve in very early larval stages in tadpoles, regeneration was followed by a complete restoration of normal motor function. In this respect the results in these very young larvae were consistent with those reported by Weiss ('36, '41) to follow regeneration of nerves of the limbs in amphibians.

5. In order to account for the complete recovery of normal muscular coordination in the youngest cases as well as the tendency toward readaptation in the older larvae it is necessary to assume the existence of myotypic specificity among the fibers of the oculomotor nerve. Possibe explanations of the foregoing findings are discussed along with their significance for the problem of the ontogenetic organization of neuronal relations in the vestibulo-ocular reflex arc.

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